SYNOPSIS OF RESEARCH PROJECT

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4.	**Name of the Degree Submitted for**	**:**	Ph.D. (Agri.)
5.	**Major Subject**	**:**	Genetics and Plant Breeding
6.	**Minor Subject**	**:**	Molecular Biology and Biotechnology
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8.	**Title of Research Problem**	**:**	Assessment of molecular and genetic diversity in Spanish bunch Groundnut (*Arachis hypogaea* L.) germplasm for dual-season adaptability and resistance to Stem Rot (*Sclerotium rolfsii*) using SSR Markers

9. **Introduction:**

Groundnut, sometimes known as "the unpredictable legume," are earthnuts, peanuts, monkey nuts, and manilla nuts. Because of its high nutritional content, this Brazilian native is a great food crop for reducing malnutrition. It is the most necessary oilseed crop ranking fourth and the thirteenth most important food crop worldwide. It is known as the "Wonder nut," "King of Oilseeds," or "Poorman's Cashew nut."

Groundnut (*Arachis hypogaea* L.) is a significant oilseed crop due to its abundance of digestible proteins, vital fatty acids, vitamins, and micronutrients. The common name for it is "peanut." According to botanical terminology, the word "arachis" refers to legumes and hypogaea means that are found below ground, suggesting that pods originated under soil. It is an allotetraploid (2n=4x=40) crop that is largely self-pollinating, a member of the Fabaceae family, and has a 2.7 Gb genome (Bertioli *et al*.,2019). The peanut is an annual herbaceous plant that reaches a height of 39 to 50 cm. The pinnate, opposite leaves have four leaflets that range in length from 1

to 7 cm and width from 1 to 3 cm. Nyctinastic, or "Sleep" motions, cause the leaves to close at night.

A relatively rare spontaneous chromosomal duplication following the hybridization of two ancient diploid species, *Arachis duranensis* (A-genome) and *Arachis ipaensis* (B-genome), along the course of evolution (Favero *et al.*,2006 and Samoluk *et al.*,2019). Phylogenetic analyses based on intron sequences and microsatellite markers validate this theory (Moretzsohn *et al*.,2013). Although the crop originated in South America, it is now produced in more than 108 countries worldwide. It is likely that the crop was brought to India in the middle of the nineteenth century (Seshadri, 1962). Runner, Virginia, Spanish, and Valencia peanuts are the four main types of peanuts, reported by the National Peanut Board.

In India, groundnuts are a significant oilseed crop that ranks second on behalf of production after soybeans and first in area. Groundnuts were grown on 32.7 million hectares worldwide in 2023–2024, with yielding on average of 1648 kg/ha and a total production of 54.31 million tons (FAOSTAT, 2024). With 19.27 million tonnes, China leads the world in groundnut output, followed by India with 10.30 million tonnes. However, India is placed 12th in terms of output, while the USA is ranked first (4469.26 kg/ha). With 19.27 million tonnes, China leads the world in groundnut output, followed by India with 10.30 million tonnes. However, India is placed 12th on output term, while the USA is ranked first (4469.26 kg/ha).

In 2023–2024, India's total groundnut area was 4.88 million hectares, and its production and productivity were 10.289 million tons and 1847 kg/ha, respectively. Approximately 90% of India's groundnut area is found in six states: Gujarat, Rajasthan, Tamil Nadu, Andhra Pradesh, Karnataka, and Maharashtra. In 2023–2024, Rajasthan produced 2.127 million tonnes of groundnuts over an area of 0.86 million hectares. Gujarat is the state that produces the most groundnuts and ranks second in terms of productivity, behind Rajasthan (Groundnut Outlook, 2025). The majority of developing countries still have low peanut yield because of several production constraints, particularly biotic and abiotic stressors.

Groundnuts are grown on over 31 million hectares in India as a rainfed kharif crop. With around 40.5% of the country's total groundnut production, Gujarat stands out among the major producing states, which include Andhra Pradesh, Karnataka,

Maharashtra, Tamil Nadu, Madhya Pradesh, Rajasthan, and Odisha. Gujarat recorded 4.16 million tonnes of groundnuts from 2.16 lakh hectares in 2022 alone, with the Saurashtra area acting as a crucial agricultural zone (Government of India, Ministry of Agriculture & Farmers Welfare, 2023).

Groundnut production is significantly threatened by soil-borne pathogens because the pods develop in close contact with the soil. Among these, stem rot—also referred to as sclerotium rot or white mold—is a highly destructive disease found in all major groundnut-growing regions. It is caused by the fungus *Sclerotium rolfsii*, which thrives in warm and moist conditions. In severely affected fields, yield losses may reach up to 80%. The occurrence and severity of the disease depend on factors such as the cropping season, sowing time, and environmental conditions. The pathogen infects plants through hyphae or germinating sclerotia, invading roots, stems, and pods. As the disease progresses, infected plants show yellowing of leaves, followed by the formation of sclerotia on affected tissues, eventually leading to wilting and plant death. In addition to reducing yield, infection by *S. rolfsii* also negatively impacts seed quality by lowering dry weight and oil content, thereby diminishing the overall value of both pods and fodder.

Multivariate analysis is the collective term for statistical techniques used to analyze data coming from several variables. Principal component analysis (PCA) and cluster analysis are two of these techniques. PCA looks for distribution patterns and eliminates duplicates from datasets after determining genetic diversity by comparing or contrasting genotypes (Dudhe *et.al*, 2020).

Evaluating genetic variation using agro-morphological traits can be difficult and time-consuming, especially if variability is unknown beforehand. Furthermore, it frequently turns out to be incorrect because of important contextual considerations (Ikram *et al*. 2020). The fact that yield depends on other factors complicates this complex quantitative characteristic (Ibrar *et al*. 2024). Crop improvement requires a diverse pool of germplasm because it minimizes screening efforts and encourages efficient allele accumulation (Darvishzadeh *et al*. 2010). It is crucial to evaluate diversity in groundnuts using marker-assisted selection and germplasm characterization.

Although visual traits sometimes fail to distinguish closely related cultivars, DNA markers, which are unaffected by epistasis and environmental factors, provide reliable insights into genetic diversity and polymorphism (Zeinalzadeh-Tabrizi *et al*. 2018). Estimating genetic diversity using molecular markers is crucial in groundnut breeding, where accurate paternal line identification is crucial (Ahmad *et al*. 2021).

Considering the importance of groundnuts as an oilseed crop and the need to provide food security, it is imperative to improve their quality traits through efficient breeding techniques in order to reach their full genetic potential. Understanding the population's genetic variability is crucial before beginning any breeding effort. It is essential to take benefit of the genetic variation that is available for cultivar development through hybridization and selection in order to adapt to different climatic conditions. Understanding many genetic characteristics, such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, and genetic advancement, determines the success of selection. By using heritable components of variation, heritability estimates help enhance traits. Information on heritability combined with genetic advancement is more helpful than information on heritability alone when evaluating the projected genetic gain in the characteristic for the choosing the best genotypes from the base population.

The performance of several characteristics known as yield components controls the complicated trait of yield. Plant breeders must understand the direction and strength of correlation between different features in order to maximize crop yield potential and achieve the aim of higher production. Path analysis divides the correlation coefficient into direct and indirect effects to measure the relative contribution of each component towards yield, whereas correlation is a biometrical method that establishes the strength of the association between two pairs of characters and offers trustworthy information on those components that can be used for criterion for choosing the breeding lines in a breeding program. Therefore, indirect selection for the qualities associated with yield will be aided by correlation in conjunction with path analysis.

Morphological characterisation, biochemical characterization, and molecular markers technologies are used to assess genetic diversity; however, environmental factors make morphological and biochemical characterization less precise (Arshad *et al*. 2009, Rani *et al*. 2023). Utilizing DNA markers, which are unaffected by

environmental factors, is necessary to comprehend genetic variety (Kumar *et al*. 2021, Rani *et al*. 2023). The genetic divergence of groundnut germplasm has been evaluated using molecular markers such as single nucleotide polymorphism (SNP), genotyping-by-sequencing (GBS), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), expressed sequence tag-simple sequence repeat (EST-SSR), and random amplified polymorphic DNA (RAPD) (Alemu *et al.* 2020). SSR markers' high polymorphism information richness, genome-wide dispersion, and reliability made them valuable for researching genetic variation and linkage mapping (Guan *et al.* 2010). SSR markers are widely utilized in population structure (PS) assessment because of their shown use and greater information richness than biallelic markers (Dreisigacker *et al.* 2005; Filippi *et al.* 2015). SSR markers have been employed by a number of authors to assess population diversity and structure. Because of their high polymorphism and codominance, SSRs are widely used in gene tagging, variety identification, diversity estimation, marker-assisted selection, and genome mapping (Ozkan *et al.* 2022).

10. Practical utility of the research project

The genetic diversity study in *Arachis hypogaea* has direct practical importance for developing improved varieties with higher yield potential. By analysing variability using agro-morphological traits and statistical tools such as Principal Component Analysis, breeders can identify superior genotypes that possess desirable characteristics like larger pod size, better seed weight, and improved productivity. This helps in selecting elite lines efficiently, reducing time and cost in breeding programs.

The project also contributes to improving nutritional security. Groundnuts are rich in proteins, healthy fats, vitamins, and essential amino acids. By identifying genetically diverse and nutritionally superior lines, breeders can develop varieties with enhanced oil quality and protein content. This is particularly beneficial in addressing malnutrition in developing countries where groundnut serves as an affordable dietary component.

This information is highly useful in research as it supports the development of effective management strategies against *Sclerotium rolfsii* by improving understanding of its infection process and environmental preferences. It aids plant breeders in identifying and developing resistant groundnut varieties, while also helping researchers

study disease epidemiology to predict outbreaks based on factors like sowing time and climatic conditions. Additionally, it guides the improvement of agronomic practices such as crop rotation, irrigation management, and field sanitation to reduce disease incidence. The knowledge of its impact on yield and seed quality further assists in assessing economic losses and designing strategies to maintain productivity, while also encouraging research into biological control methods using antagonistic microorganisms for sustainable disease management.

In addition, the use of molecular markers such as Simple Sequence Repeat provides a highly reliable method for assessing genetic diversity and population structure. Unlike morphological traits, these markers are not influenced by environmental conditions, making them valuable for accurate identification of varieties, germplasm conservation, and marker-assisted selection. This accelerates the breeding process and ensures precision in developing improved cultivars.

The correlation and path analysis performed in the study have practical implications in indirect selection strategies. Since yield is a major trait influenced by multiple components, understanding the relationship among traits allows breeders to focus on key contributing factors. This improves selection efficiency and helps in designing breeding programs aimed at maximizing yield.

Furthermore, the project aids in the conservation and utilization of diverse germplasm resources. By identifying genetically distant parents, breeders can create hybrids with greater heterosis (hybrid vigour), leading to better-performing varieties. This ensures sustainable crop improvement and reduces the risk of genetic erosion.

Finally, the outcomes of this research support agricultural policy and extension services. The development of high-yielding, stress-tolerant, and nutritionally rich groundnut varieties can increase farmers' income, boost oilseed production, and reduce dependency on imports. Overall, the project plays a significant role in ensuring food security, economic stability, and sustainable agriculture.

11.Objectives

The present study is planned with four environments (two seasons and two locations) the following objectives. keeping in mind the previously described viewpoints.

1. To estimate the genetic variability, heritability and genetic advance for different quantitative traits under dual season.
2. To find out the genotypic and phenotypic correlation between seed yield and yield contributing characters.
3. To study the nature of G×E interaction and stability of groundnut germplasm.
4. To access the genetic diversity using markers.
5. To validate the molecular markers associated with stem rot resistance in groundnut genotypes.

12. Review of Literature

Enhancing both quantitative and qualitative characteristics in nature is a constant focus for plant breeders. Consequently, knowledge of the genetic makeup of the numerous significant traits and their interrelationships is necessary. The following subheadings provide an overview of the literature on variability, correlation, phenotypic stability, gene introgression and marker-assisted breeding of diversity in groundnut.

12.1 Genetic variability Parameters

A biological population's variability results from the genetic makeup of its members in relation to the prevailing environments. An effective breeding program must begin with a study of genetic variability using appropriate criteria including genotypic coefficient of variation, heritability estimates, and genetic advancement.

Khan *et al*. (2020) evaluated landraces of Bambara groundnut (*Vigna subterranea* L.) to assess genetic variability, heritability, and trait association for yield improvement. The study revealed significant variation among accessions for all qualitative and quantitative traits, indicating wide genetic diversity. High coefficients of variation were observed for several traits, while strong positive correlations were found between yield and traits such as fresh pod weight, dry pod weight, and dry seed weight. High PCV, GCV, heritability, and genetic advance for biomass and yield-related traits suggested the predominance of additive gene action and effectiveness of direct selection. Cluster analysis grouped accessions into distinct clusters, with superior genotypes identified from clusters II and IV showing high yield potential. The study

highlighted the importance of genetic diversity and key yield-contributing traits for effective selection and improvement of Bambara groundnut in breeding programs.

Mitra *et al*. (2021) conducted an investigation on 31 groundnut accessions to assess genetic divergence, character association, and path coefficients using 13 quantitative traits. The study revealed high phenotypic and genotypic coefficients of variation for number of pods per plant, secondary branches, kernel width, and pod yield, indicating substantial variability among the accessions. High heritability coupled with high genetic advance was observed for pod length, pod yield, and number of pods per plant, suggesting the predominance of additive gene action for these traits. Correlation analysis indicated that number of secondary branches, kernel width, pod length, and number of pods per plant were positively and significantly associated with pod yield. The study identified five highly divergent accessions, namely TAG-24, TG-69, ICGV-02005, TG-73, and TG-80, as promising candidates for future breeding programs aimed at improving yield and genetic diversity in groundnut.

Yadav *et al*. (2023) evaluated 45 groundnut genotypes for genetic variability and trait association. Significant variation was observed for all traits. High heritability, GCV, and genetic advance were recorded for biological yield, 100-kernel weight, kernel yield, dry pod yield, and pods per plant, indicating additive gene action and high selection potential. Kernel yield, pods per plant, and biological yield showed strong positive correlations with dry pod yield and were identified as key traits for direct selection in groundnut improvement.

Sravanti *et al*. (2024) evaluated fourteen advanced breeding lines along with three check varieties of groundnut to study genetic variability, correlation, and path analysis for yield and its component traits. The analysis of variance revealed significant differences among genotypes for all the characters studied, indicating the presence of considerable genetic variability. They reported high and moderate genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for traits such as hundred pod weight, hundred kernel weight, dry pod yield, dry haulm yield, and kernel yield, suggesting good scope for improvement through phenotypic selection. Correlation studies indicated that dry pod yield per plant was positively and significantly associated with days to 50% flowering, dry haulm yield, kernel yield, and pod weight traits. Further, path coefficient analysis revealed that kernel yield, hundred

pod weight, dry haulm yield, days to 50% flowering, hundred kernel weight, initial plant count, and sound mature kernels had strong positive direct effects on pod yield per plot. The study concluded that these traits could be effectively utilized as selection criteria for improving pod yield in groundnut breeding programs.

Yami *et al*. (2025) studied genetic variability in groundnut genotypes. Significant differences were observed for most traits, with high PCV and GCV indicating good selection potential. Kernel yield correlated positively with most traits, and high heritability with genetic advance was noted for key yield components. Cluster and principal component analyses revealed substantial diversity, suggesting opportunities for breeding and improvement.

12.2 Correlation coefficient

Although it serves as a foundation for selection, the understanding of the relationship between yield and its components is extremely valuable to breeders. It is commonly recognized that various yield components frequently show a significant degree of correlation with yield and with each other. Therefore, gathering the best possible combination of yield-contributing traits in a single genotype would be made easier with an understanding of character correlation. Galton introduced the idea of correlation in 1889, and Fisher expanded on it in 1918. A review of previous research on the correlation coefficient between groundnut production and its contributing characteristics has been attempted.

Khan *et al*. (2020) examined in their study of 150 accessions, found moderate PCV and GCV estimates for traits such as shelling percentage (8.4, 6.7) and hundred seed weight (12.3, 10.4), high for fresh pod weight (33.03, 33.12) and dry pod weight (30.1, 30.14), moderate heritability for pod yield plant-1, high for 50% flowering, high heritability in dry pod weight (g) (99.75) and fresh pod weight (g) (95.2). They found that the total number of pods per plant was significantly positively correlated with the weight of the dry pods, fresh pods, hundred seeds, and shelling %.

Abdurrasheed *et al*. (2021) studied 15 groundnut genotypes for genetic variability and character association studies. They discovered that while other variables had a high heritability but a low genetic advance, the number of pods plant-1 showed a high heritability (84.87%) and a high genetic advance (59.63%). They observed that estimates of GCV and PCV were high for protein, carbohydrates, and the number of

pods and seeds per plant. Additionally, they discovered a strong positive association between the number of pods per plant (0.76 to 0.99) and every agronomic characteristic, with the exception of 100 seed weight (-0.36).

Shrotri *et al*. (2021) studied that ICGV00189, ICGV0213, and ICGV00202 demonstrated high mean performance for pod yield and the related traits in an experiment to evaluate genetic diversity for pod yield and component properties. Thirty groundnut genotypes for ten features were analysed. Kernel yield plant-1 (g) (33.16, 36.05), pod yield plant-1 (g) (30.30, 33.47), shelling percentage (8.35, 9.60), and oil content (2.53, 3.05) had the greatest GCV and PCV values. The genotypes ICGV02038 and ICGV02005 were found to have high content (46.3%) and high heritability for pod yield plant-1 (82.3), kernel yield plant-1 (84.5), shelling percentage (74.5), and oil content (74.1).

Killi and Beycioğlu (2022) examined the variability of 10 groundnut varieties' pod yield, oil, and protein content. They discovered that shelling percentage (95.4%), hundred seed weight (91.6%), and hundred pod weight (88.3%) had high heritability estimates. Protein content (2.6 and 3.8%) and oil content (2.0 and 2.8%) had low GCV and PCV values.

Madhuri *et al*. (2022) observed low estimates of PCV for days to flowering (5.2), 100 seed weight (10.1), and pod yield per plant (11.1) in their study of twenty advanced backcross lines in BC2F6 and BC1F6. Out of all the traits, oil content has the highest heritability (91.2). Additionally, they reported that plant height showed high GCV (20.21), high heritability (91.2), and GAM (39.77). They found a substantial and positive association between pod yield plant-1(g) and kernel yield plant-1(g), plant height, number of pods plant-1, and number of secondary branches plant-1.

Yashaswini *et al*. (2024) examined the F6 and F7 families of three crosses: GKVK-16 × KCG-2, GKVK-13 × KCG-2, and GKVK-6 × KCG2. They also checked KCG-6, KCG-2, and TMV-2 to determine high-yielding and LLS disease-resistant lines. They discovered that the cross GKVK -6 × KCG -2 had high PDI values at day 120, indicating the lines more impacted by LLS illness. Additionally, they reported that in the cross GKVK-16 × KCG-2, there were high values of GCV and PCV, high heritability combined with high GAM for PDI at 60 DAS (23.4 and 25.33), PDI at 90

DAS (23.52 and 28.93), and PDI at 120 DAS (36.34 and 51.73). They discovered that the GKVK-13 × KCG-2 hybrid had 85% heritability for PDI at 90 DAS.

Sravanti *et al*. (2024) evaluated fourteen advanced breeding lines along with three check varieties of groundnut to study genetic variability, correlation, and path analysis for yield and its component traits. The analysis of variance revealed significant differences among genotypes for all the characters studied, indicating the presence of considerable genetic variability. They reported high and moderate genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for traits such as hundred pod weight, hundred kernel weight, dry pod yield, dry haulm yield, and kernel yield, suggesting good scope for improvement through phenotypic selection. Correlation studies indicated that dry pod yield per plant was positively and significantly associated with days to 50% flowering, dry haulm yield, kernel yield, and pod weight traits. Further, path coefficient analysis revealed that kernel yield, hundred pod weight, dry haulm yield, days to 50% flowering, hundred kernel weight, initial plant count, and sound mature kernels had strong positive direct effects on pod yield per plot. The study concluded that these traits could be effectively utilized as selection criteria for improving pod yield in groundnut breeding programs.

Mudgalkar *et al*. (2025) evaluated 61 groundnut genotypes for genetic variability, correlation, and path analysis. Significant variability was observed, with kernel yield per plant showing strong positive associations with 100-pod weight, 100-kernel weight, pod yield, shelling percentage, and maturity traits. Path analysis revealed kernel yield, pod yield, harvest index, and biological yield had the highest direct effects on dry pod yield, while other traits contributed indirectly. The study highlights key yield-contributing traits for selection in breeding high-yielding groundnut cultivars.

12.3 Stability analysis

The breeding program requires information on the degree of genotype × environment interaction for yield and component traits in order to generate high-yielding, phenotypically stable varieties. The phrase "genotype-environment interaction" refers to the interaction of genetic and non-genetic influences on character development. G × E interaction quantifies the variations in a genotype's reaction to environmental changes. To get unbiased estimates of genetic variance, it is crucial to detect and assess the amount of the G × E interaction. The very success of a crop

improvement program connected to consistent genotype performance in a variety of settings is based on the absence of G × E interaction. A methodology to gauge genetic adaptability is called the stability approach. The capacity of a genotype to produce a comparatively small range of phenotypes in various settings is known as adaptability. Various workers have employed different stability measures. Finlay and Wilkinson (1963) used the slope of linear regression as a stability metric. When evaluating the phenotypic stability of a genotype, Eberhart and Russell (1966) recognized the need of taking into account both the linear (bi) and nonlinear (S^2di) components of the genotype x environment interaction.

The following is an overview of some of the previous groundnut stability analysis reports:

Barad (2021) carried out a study to examine the stability analysis of fifty groundnut genotypes. The mean squares owing to environments (linear) were significant for every character, whereas the G x E (linear) component was significant for every attribute except shelling out-turn. Due to their high pod yield per plant, genotypes JVB-2516 and JVB-2529 were average stable.

Sari *et al*. (2021) carried out a study to examine the stability analysis of seven groundnut genotypes. The BBL 1.1 line is the variety with an earlier harvest period, the largest yield potential, good stability, and broad adaptability, according to the stability and adaptability analysis. The production lines CCC 1.1, PWBG 6, PWBG 5.2.1, and SS 2.4.2 have poor productivity but are stable in all experimental settings. In a production setting, the SS 3.4.2 is appropriate for planting. For production in marginal habitats, like drought circumstances, TVSU 86 is perfect.

Kumari *et al*. (2022) conducted stability analysis research on eighteen groundnut genotypes. Days to maturity and pod production per plant were shown to be significantly impacted by the genotype x environment (G x E) interaction. For each of the six traits under study, both linear and non-linear environmental components were found to be extremely significant.

Fadakar *et al*. (2023) conducted stability analysis research on eleven groundnut genotypes. Based on all non-parametric techniques, the ideal genotype selection indicator (IGSI) results showed that the most stable genotypes were 178, 128, 201, 176, and 115, which had the highest IGSI values.

Gangadhara and Gor (2023) carried out an experiment to investigate the stability analysis of nine groundnut genotypes. In the combined ANOVA, the G × E interaction had a substantial impact on dry pod yield and its components. "Mean vs. stability" revealed that the most valued and stable genotypes were variety TAG 24 and interspecific derivative NRCGCS 446. While NRCGCS 254 produced more pods in Mohanpur, GG 7 produced more in Junagadh.

Umata and Hunduma (2024) conducted stability analysis research on twelve groundnut genotypes. The mean squares of the analysis of variance for seed yield per hectare revealed highly significant genotypes and G x E interactions. Genotype accounted for 43.73% of the total variation, whereas environmental factors accounted for 53.25%. The biplot accounted for 84.93% of the total variation. The AMMI model identified 19748 and 19750 as the most stable models. These genotypes are therefore considered to be the most appropriate for the research sites.

Wang *et al*. (2024) conducted stability analysis research on 52 groundnut genotypes. Nuclear magnetic resonance, gas chromatography, and a N analyser were used to determine the protein, oil, and fatty acid composition of the harvested seeds, respectively. Significant influences on the examined attributes were assessed for accession, the FAD2 gene (FAD stands for fatty acid desaturase), location, year, and their interactions. While the average protein content of accessions grown in Florida was significantly lower than that of accessions cultivated in Georgia or New Mexico, the average oil content of accessions grown in Florida or Georgia was much greater than that of accessions grown in New Mexico. Given the negative relationship between oil and protein concentration, this is to be expected.

2.4 Genetic diversity using molecular markers

Banla *et al*. (2020) evaluated 94 groundnut (*Arachis hypogaea* L.) accessions using agro-morphological traits and SNP markers. High variability was observed for yield, number of pods per plant, and late leaf spot incidence. Principal component analysis identified hundred seed weight, maturity, and disease incidence as major contributors to variation. Number of pods per plant showed strong positive association with yield. SNP analysis revealed moderate genetic diversity with minimal geographical differentiation. The genotypes were grouped into three clusters, indicating useful variability for breeding.

Akohoue *et al*. (2020) analyzed 281 Kersting's groundnut (*Kerstingiella geocarpa*) accessions for genetic diversity and marker-trait associations using 493 SNPs. Population structure revealed two subpopulations and four clusters differentiated by seed coat colour (Fst = 0.37–0.79). GWAS identified 10 significant SNP-trait associations, six stables across environments. Genomic selection showed moderate to high prediction accuracy for key yield and phenology traits. The study demonstrates substantial genetic variation and potential for breeding improvement in Kersting's groundnut.

Radhakrishnan *et al*. (2021) reviewed the genetic improvement of cultivated groundnut (*Arachis hypogaea* L.), a tetraploid crop derived from *A. duranensis* and *A. ipaensis*. Breeding has focused on higher yield, seed traits, and resistance to foliar diseases, viruses, and nematodes. Advances over the last 15 years include mapping populations, QTL identification, draft genomes, and the 58K Axiom_Arachis SNP array, enabling marker-assisted and genomics-assisted breeding. In India, over 200 improved cultivars have been released, and ongoing genomics programs aim to develop high-yielding, stress-tolerant varieties without compromising quality.

Ellandula *et al*. (2025) assessed genetic diversity in 50 groundnut (*Arachis hypogaea* L.) genotypes using morphological traits and 50 SSR markers. Subpopulations I and II showed a net nucleotide distance of 0.32, with highest heterozygosity in subpopulation II (0.38) and low Fst in subpopulation I (0.35). Principal component and cluster analyses grouped the genotypes into two clusters, with the first four components explaining 74.45% of variation. Principal coordinate analysis supported the clustering, accounting for 22.64% of variation. Genotypes COG0537, COG18032, and COG18040 were identified as promising parents for improving oil content and yield, while COG15033 was a high-yielding line with high oleic acid. The study demonstrates the value of SSR markers and morphological evaluation for selecting diverse parental lines in groundnut breeding.

2.5 Molecular markers associated with stem rot resistance.

Pramanik *et al*. (2019) conducted a genetic assessment of 90 germplasm lines and six groundnut varieties using 13 morphological traits and 125 Simple Sequence Repeat (SSR) markers. Among the 125 molecular markers, 26 were found to be polymorphic, generating a total of 105 alleles. The study reported genetic diversity

ranging from 52% to 83%, with Polymorphic Information Content (PIC) values between 0.46 and 0.81, and a mean PIC of 0.42, indicating a relatively high level of genetic diversity among the tested genotypes. Significant positive correlations were observed, including between hundred-kernel weight and hundred-pod weight (r = 0.769), and between hundred-kernel weight and kernel yield (r = 0.899). Additionally, sound mature kernel and pod weight showed strong positive associations with kernel yield, kernel weight, and harvest index.

Dodia *et al*. (2019) conducted linkage analysis and quantitative trait locus (QTL) mapping for stem rot resistance in a bi-parental recombinant inbred line (RIL) population of 270 individuals derived from TG37A (susceptible) × NRCG-CS85 (resistant). Using a Genotyping-by-Sequencing approach, they generated single nucleotide polymorphism (SNP) data, which enabled the construction of a genetic map comprising 585 SNP loci spanning 2430 cM. Multi-season phenotyping and genotyping-based QTL analysis did not reveal any major main-effect QTLs but identified 44 significant epistatic QTLs, explaining 14.32% to 67.95% of the phenotypic variation. The authors suggested that these identified genomic regions could be further validated and utilized for marker development, facilitating genomics-assisted breeding to develop stem rot-resistant groundnut varieties. Cui et al. (2020) identified resistant and susceptible bulks from a recombinant inbred line (RIL) population and applied QTL sequencing (QTL-seq) to detect genomic regions associated with stem rot resistance in peanut. They identified two QTL regions controlling resistance and developed SNP markers linked to these QTLs. The markers were validated through a blind selection test in a subset of the population not used in the initial analysis. Additionally, the lines selected for bulk sequencing demonstrated a clear and strong separation in stem rot resistance.

Guclu *et al*. (2020) screened groundnut genotypes and wild Arachis species over two years using an SSR marker associated with fungal resistance. In the first year, significant variation was observed among the genotypes for disease-related traits, with disease scores ranging from 2 to 5 and a general mean of 4.7. They reported that 5.7% of the collection were moderately susceptible, while 1.6% were moderately resistant. In the second year, these selected genotypes were evaluated under similar field conditions. The results indicated that the lowest disease scores were observed in genotypes ACG 14 (subsp. fastigiata var. vulgaris) and ACG 101 (subsp. hypogaea var.

hypogaea). Among the wild Arachis species, the average disease score was 3.7, with A. batizocoi showing the lowest score of 2.5, followed by A. duranensis and A. cardenasii. The study found no correlation between SSR marker data and field phenotyping, leading the authors to recommend the development of new validated resistance-related markers for more accurate selection of resistant and susceptible genotypes in future studies.

Hawaladar *et al*. (2022) recorded disease incidence ranging from 10.71% to 18.50% across 100 fields and collected ten disease samples per site, with each sample representing a unique isolate. Pathogenicity was assessed by inoculating all ten isolates onto three groundnut varieties—TMV-2, GKVK-5, and GKVK-27—all of which were successfully colonized. The fungal isolates displayed cultural and morphological characteristics consistent with *Sclerotium rolfsii*. Sequence analysis of the ITS and LSU regions of the ten isolates revealed maximum nucleotide identities of 90.10–98.80% with previously reported *S. rolfsii* isolates from the NCBI database, while the SSU region showed nucleotide identities ranging from 96.8% to 97.8%.

13. Materials and methods

The experimental material will be comprising 40 groundnut genotypes and 5 checks i.e., GG-39, GG-35, GG-36, GG-40 and GG-37 will be selected for further study. All genotypes will be evaluated during two seasons and at two locations i.e.,

13.1	**Location**	:	Main Oilseed Research Station, JAU DFRS, Rajkot
13.2	**Year and Season of Experiment**	:	*Kharif* 2026 *Rabi* 2027
13.3	**Experimental Design**	:	Randomised Block Design.
13.4	**No. of Replications**	:	Three
13.5	**Spacing**	:	45 x 15 cm

13.7 List of Genotypes

1. JB-1865	**11. J-126**	**21. J-118**	**31. JB-1850**
2. J-120	**12. JB-1614**	**22. J-127**	**32. JB-1853**
3. JB-1644	**13. JB-1632**	**23. J-128**	**33. JB-1854**
4. J-125	**14. JB-1842**	**24. JB-1598**	**34. JB-1855**
5. JB-1787	**15. JB-1849**	**25. JB-1631**	**35. JB-1855**
6. JB-1787	**16. JB-1857**	**26. JB-1643**	**36. JB-1868**
7. JB-1873	**17. JB-1856**	**27. JB-1662**	**37. JB-1868**
8. JB-1857	**18. JB-1859**	**28. JB-1664**	**38. JB-1589**
9. JB-1851	**19. JB-1642**	**29. JB-1687**	**39. JB-1638**
10. JB-1857	**20. JB-1638**	**30. JB-1842**	**40. ICJB-201214**

13.8 Observations to be recorded

Except days to 50% flowering and days to maturity, which were recorded on a plot basis, observations will be made for each character individually by selecting five competing plants in each genotype in each replication. These are particulars of the recorded data.

13.8.1 Days to 50% flowering

Number of days from the date of sowing to date on which flowers appeared in 50**%** plants in the plot will be recorded.

13.8.2. Days to maturity

Number of days taken from sowing to 80% maturity. The total number of days will be calculated from sowing to the maturity of all plants in the plot.

13.8.3. Plant height (cm)

Height will be measured in centimetre from ground level to the tip of main axis at harvesting time.

13.8.4. SPAD chlorophyll meter reading (SCMR) at 45 DAS

SPAD chlorophyll meter reading (SCMR) will be measured on five randomly selected plants from each genotype in each replication at 45 DAS using chlorophyll meter. The measurements will be taken on the third leaf from the terminal bud of the main axis.

13.8.5. Pod yield per plant (g)

The weight of total number of pods per plant after harvesting will be recorded and expressed in gram.

13.8.6. 100-pod weight (g)

Randomly taken 100 pods from each entry will be weighed on electric balance in grams.

13.8.7. Kernel yield per plant (g)

The weight of the kernels shelled from manual crushing of mature pods per plant will be recorded and expressed in grams.

13.8.8. 100-kernel weight (g)

Hundred seeds counted from random sample from each plot will be weighed in grams on electric balance.

13.8.9. Seed length(mm)

Ten mature seeds were selected randomly from each plant in each replication and seed length will be recorded in millimetre.

13.8.10. Sound mature kernels (%)

Fully matured and immature kernels counted from representative sample obtained from each plot and expressed as percent sound matured kernels.

13.8.11. Biological yield per plant (g)

After harvesting and sun drying, all the selected plants will be weighed in grams and averaged.

13.8.12. Shelling percentage (%)

Pod weight will be recorded from each plant in grams. Then, the weight of kernels after shelling the pods of same plant will be recorded in grams. The shelling percentage will be calculated as

$$\textbf{Shelling percentage} = \frac{\text{kernel weight (g)}}{\text{Pod weight}} \times 100$$

13.8.13. Harvest index (%)

The biological yield (total dry matter after harvesting and sun drying) and seed yield each plant will be recorded in grams and harvest index will be calculated as under: -

$$\textbf{Harvest Index (\%)} = \frac{\text{Pod yield (g)}}{\text{Biological yield (g)}} \times 100$$

13.8.14. Specific leaf area (cm^2/g)

The fully expanded, healthy third leaf (in case of damage, fourth leaf) from the apex of the main axis in each plant will be collected in the plastic bags at 80 days after sowing (DAS) in morning hours and soon brought to the laboratory. The leaf area of the four leaflets will be measured using a leaf area meter after which the leaves will be oven dried at 800°C for 48 hours to determine the leaf dry weight. SLA will be computed using the following formula:

$$\textbf{SLA} = \frac{\text{Leaf area } (cm^2)}{\text{Dry leaf weight (g)}} \times 100$$

13.8.15. Root diameter (mm)

Root diameter will be measured in millimetre at collar region from five plants of a genotype by using digital vernier calliper.

13.8.16. Specific root length (SRL)

Specific root length will be calculated with an equation of Zobel (2005) and expressed in cm per gram. SRL (cm g-1) =Root length (cm)/Root dry weight (g)

$$\textbf{SRL (cm } g^{-1}) = \frac{\text{Root length (cm)}}{\text{Root dry weight (g)}}$$

13.8.17 Stem rot incidence (%)

Percent Disease incidence (%) at the Maturity The total number of plants per row and number of affected plants in the same row will be counted and percent disease incidence will be calculated by following formula at 60 DAS, 90 DAS and maturity separately.

$$\textbf{Percent Disease incidence (\%)} = \frac{\text{Number of healthy plants in a row}}{\text{Total number of plants in a row}} \times 100$$

14. Statistical analysis

The mean values of all the genotypes for all the characters will be subjected to statistical analysis in the following aspects:

14.1. Analysis of variance for experimental design

The experiment will be done in Randomised Block Design (RBD) with three replications. The analysis of variance will be carried out for individual as well as over the environments as per standard procedure given by Panse and Sukhatme (1985).

14.2. Variability estimates

The method recommended by Burton (1952) will be used to measure the variability parameters, namely the genotypic and phenotypic coefficients of variation. The method recommended by Allard (1960) will be used to calculate the heritability and genetic advancement.

14.3. Correlation coefficients

The procedure described by Hazel (1943) will be used to determine the genotypic and phenotypic correlations.

14.4. Stability analysis

The stability parameters of genotypes will be computed as per the method suggested by Finlay and Wilkinson (1963) and further amplified and generalised by Eberhart and Russell (1966).

14.5 Disease rating scale for stem rot disease incidence (%)

Disease rating scale for stem rot disease incidence will be followed as outlined by Ashok *et al*., (2004).

Scale*	Disease reaction
0%	Immune
1-10%	Highly resistant
10-20%	Resistant
20-50%	Moderately resistant
>50%	Highly susceptible

* Percent of infected plants per row

14.6. Molecular Markers

Simple Sequence Repeat (SSR) primers synthesized will be used in the study.

14.7. Isolation of genomic DNA.

Genomic DNA was extracted from tender leaves collected from 15-20 days of plant grown in field following the CTAB method (Saghai *et al*.,1984).

14.8. Quantification and visualisation of DNA

DNA will be quantified by measuring optical density (O.D.) at A260 and A280 with a Nanodrop Spectrophotometer (ND2000). The gels were photographed under a Gel Documentation system (Perkin Elmer Geliance 200).

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